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Gross Morphological and Histological Features of Larynx in Yankasa Sheep (*Ovis aries*)

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ABSTRACT

The present study, conducted on the larynx of five adult Yankasa sheep was aimed at exploring its basic anatomy and histological features. The larynx consists of four cartilages; the unpaired epiglottis rostroventral, unpaired thyroid ventrally, dorsal paired arytenoid, and caudal unpaired cricoid. These cartilages presented distinct morphological features and were connected to each other by ligaments. The intrinsic laryngeal muscles include; the dorsal cricoarytenoid and transverse arytenoid muscles. The lateral cricoarytenoid was concealed by the thyroid laminae. The ventrally located thyroarytenoid and cricothyroid muscles. The laryngeal cavity comprised of rostral vestibule, a narrow middle glottic cleft and a wide infraglottic cavity caudally. Histologically, the epiglottis was lined by stratified squamous keratinized epithelium, the thyroid cartilage was lined by pseudostratified columnar epithelium, whereas the arytenoid cartilage was lined by pseudostratified columnar ciliated epithelium with goblet cells. The irregular loose connective tissue of the propria submucosa comprised of connective tissue cells mainly fibroblast, and fine blood capillaries, whereas the deeper part presented glandular tissues, ducts, fat cells and cartilages. It is envisaged that the study has provided basic information on gross and histological feature of the larynx in the Yankasa sheep.

Keywords: Histology, Laryngeal cartilages, Laryngeal muscles, Yankasa Sheep

INTRODUCTION

The larynx is a bilaterally symmetrical, tube-shaped musculo-cartilaginous organ that connects the pharynx to the trachea (Konig and Liebich, 2004). It plays a vital role in phonation, regulation of airflow through its lumen, and protection of the lower airway during swallowing by preventing aspiration of foreign material (Parkash and Kumar, 2019). In addition, the laryngeal cavity contains lymphoid tissue in the form of nodules within the mucosa, which are major components of the mucosa-associated lymphoid tissue capable of establishing a local immune response (Casteleyn, *et al*., 2008). Gross anatomy and histology of the larynx have been documented in giraffe (Erdogan and Perez, 2013), large ruminants, and donkey (Eshra *et al*., 2016; Metwally *et al*., 2018), and pigs (Parkash and Kumar, 2019). However, studies on gross anatomy and histological features of the larynx in Yankasa sheep are few in available literatures. Thus, the present work was planned to elucidate on the gross morphology of the laryngeal cartilages, muscles, cavities as well as histological details of the laryngeal mucosa and cartilages in Yankasa sheep. Knowledge of larynx anatomy is essential to plan for surgical intervention or clinical investigation (Metwally *et al*., 2018), and for a better understanding of pathogenesis, fate and development of certain diseases condition associated with the larynx (Thiemann and Bell, 2001; Badawy, 2005).

MATERIALS AND METHODS

The present study was conducted on five (5) heads of adult local mixed breed sheep (Yankasa) of both sexes. The heads, decapitated at the occipito-atlantal joint, were obtained from Maiduguri Central Abattoir immediately after routine slaughter and immediately fixed in 10% formalin for approximately 3 days. The head samples were processed at the Veterinary Gross Anatomy Laboratory, University of Maiduguri as described below:

Gross Anatomical Studies

The mandibles were disarticulated and detached from the heads to expose the laryngeal region. The larynx comprising of the intrinsic muscles and cartilages were identified and carefully excised out. The gross morphology and topographical features of laryngeal cartilages, muscles and cavities were studied. The studied features were photographed at different anatomical planes with a digital camera (Canon 60D).

Histological Studies (Light Microscopy)

Fixed tissues collected from different portions of the larynx were processed by routine paraffin technique of light microscopy, and sections of 5-6 micrometer (µm) were cut and stained using Hematoxylin and Eosin (H and E) (Luna,

1968). Histo-architecture of all the sections were studied and photomicrographs at different magnifications were taken using a light microscope (N-180, Seeuco Electronics Technology, China).

RESULTS

Morphological Findings

A. Laryngeal Cartilages

The larynx in Yankasa sheep was comprised of 5 cartilages. These include; unpaired epiglottis, thyroid cartilage, cricoid cartilage and paired arytenoid cartilages (Figs 1-4).

Epiglottis Cartilage

The epiglottis was the most rostral of the laryngeal cartilages, situated ventrally at the laryngeal entrance. It was leaf-like shaped and presented two surfaces, two lateral limits, an apex and a base. The laryngeal surface was convex rostro-caudally and slightly concave transversely. The concavity of the laryngeal surface was shallow towards the apex. The lingual surface was smooth and deeply convex transversely and slightly concave rostro-caudally. The ventral surface presented several mucosal folds and was strongly concave. The apex which is the free surface of the epiglottis constituted the ventro-rostral limit of the laryngeal inlet, whereas the base possesses a connecting stalk in form of a rounded mass (petiolus) that articulates via ligaments with the notch formed by the thyroid cartilage. The two lateral boundaries of the epiglottis presented the aryepiglottic folds (Figure 1 A-B).

Thyroid Cartilages

It was the largest and articulated with all the cartilages. It presented an irregular quadrilateral outline on a lateral view. The thyroid cartilage comprises of right and left laminae, which constitute the body of the thyroid. These laminae were separated by wide space dorsally, joined ventrally, presenting a V-shape orientation on cranio-caudal view. The mid-junction of the thyroid laminae internally had a deep linear fossa. A ventral laryngeal prominence existed towards the caudal end of the mid-ventral union of the laminae. The laminae presented dorsal, rostral and caudal borders. The dorsal border was relatively straight where it terminated into relatively short rostral and long caudal cornua. The rostral border was convex dorsally, then sloped toward the mid-ventral portion to form the dorsal fissure and rostral thyroid notch. The dorsal fissure at the caudal border was deep whereas the ventral caudal thyroid notch was shallow for articulation with the arch of the cricoid cartilage (Figures 2 & 3).

Arytenoid Cartilages

The paired arytenoid cartilages capped the wide dorsal surface formed by the laminae of the thyroid cartilages, thus constituting the main roof of the larynx. These cartilages articulate caudally with the cricoid lamina. The arytenoid cartilages were triangular-shaped in which three processes emerged. The base was medially situated, while the apex was located laterally. The arytenoid processes included: the corniculate process which extended dorsomedial, a vocal process that projected ventrally into the lumen of the laryngeal cavity and, a muscular process which extended laterally. There exists an articular facet ventromedial at the caudal surface for articulation with the cricoid laminae (Figure 4 A-B).

Cricoid Cartilage

The cricoid cartilage was the most caudal of the laryngeal cartilages. It articulates caudo-dorsal with the arytenoid cartilage and caudo-ventral with the thyroid cartilage. It has a broad dorsal part that consists of right and left laminae and a narrow arch ventrally, giving the cricoid a “signet ring” appearance. The two laminae are quadrilateral shaped and united dorsally to form a median crest. The dorsal surface of the laminae provides for the attachment of muscle. There exist a craniolateral facets on the rostral margin of the laminae for articulation with the arytenoid cartilage, and a caudal facet for articulation with the thyroid cartilage. The caudal border of the cricoid laminae presented a caudal-directed blunt process (Figure 5 A-C).

B. Intrinsic Muscles of the Larynx

There were five intrinsic muscles of the larynx in the present study. Two of which were situated dorsally and these includes: the dorsal cricoarytenoid muscle and transverse arytenoid muscles. The lateral cricoarytenoid lies medially and concealed by the thyroid laminae, whereas the thyroarytenoid muscle and cricothyroid muscles were situated ventrally.

Dorsal Cricoarytenoid Muscle

These are paired V-shaped muscles that covers the dorsal surface of the cricoid laminae. It originated from the caudo-lateral margin of the cricoid laminae and the median crest of the cricoid and inserted rostro-lateral on the muscular processes of the arytenoid cartilage (Figure 6 A).

Transverse Arytenoid Muscle

It was a transversely oriented muscle that lies between the rostral portion of the dorsal cricoid laminae and the caudo-laterally directed processes of the arytenoid cartilages. A median tendinous intersection interrupted this muscle as its crosses to connect the two muscular processes of the arytenoid cartilage (Figure 6 A).

Thyroarytenoid Muscle

These are paired fan-shaped muscles that originates from the base of the epiglottis, dorso-lateral margin of rostral thyroid notch, dorsal thyroid fissure, and runs obliquely caudo-dorsal to insert on the caudo-dorsal border of the thyroid laminae adjacent to the origin of thyrohyoid muscle (Figure 6 B-C).

Cricothyroid Muscle

The paired cricothyroid muscles occupy the ventral and rostro-lateral surfaces of the cricoid arch. It arises from the left and right cricoid arches, runs cranio-dorsal to insert on the caudal border of the left and right thyroid laminae, partially covered by the thyrohyoid muscle (Figure 6 B-C).

C. Laryngeal Cavity

The laryngeal cavity was divided into three portions; rostral narrow vestibule, middle and wide caudal infraglottic cavity caudally. The vestibule comprises of laryngeal inlet bounded by thyrohyoid muscle and the epiglottis rostroventrally, aryepiglottic folds laterally, and corniculate process of the arytenoid caudo-dorsally. The glottic cleft was the narrow median portion bounded dorsally by the adjacent paired arytenoid cartilages and the vocal fold, whereas the wide caudal infraglottic cavity connects to the trachea. (Figure 7 A-C).

The vestibule on a median section was compacted and extends ventrally into the median laryngeal recess. This recess was obliquely shallow depression that extend from the base of the epiglottis to vocal fold (Figure 8). A vestibular fold was present between the rostral silt-like opening of the lateral laryngeal ventricle and the vocal fold caudally. Immediately caudal to the laryngeal recess, there was a short ventrally directed depression which marked the rostral limit of the vocal fold. The vocal fold was short and extend caudo-dorsal from the floor of the larynx to unite dorsally with the arytenoid cartilage (Figure 8).

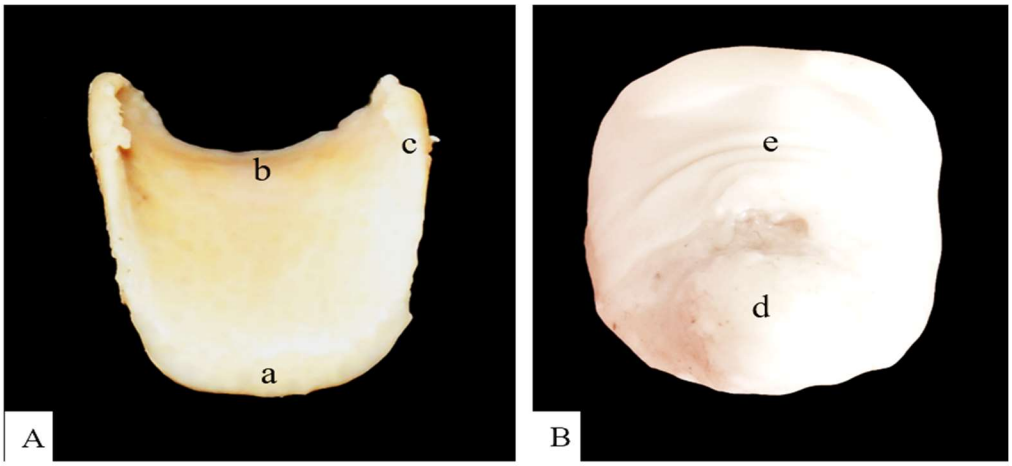

Figure 1: Epiglottic cartilage of Yankasa sheep; A- rostro-dorsal view; B- ventral view showing; (a) apex, (b) base, (c) aryepiglottic fold, (d) petiolus , (e) mucosal fold.

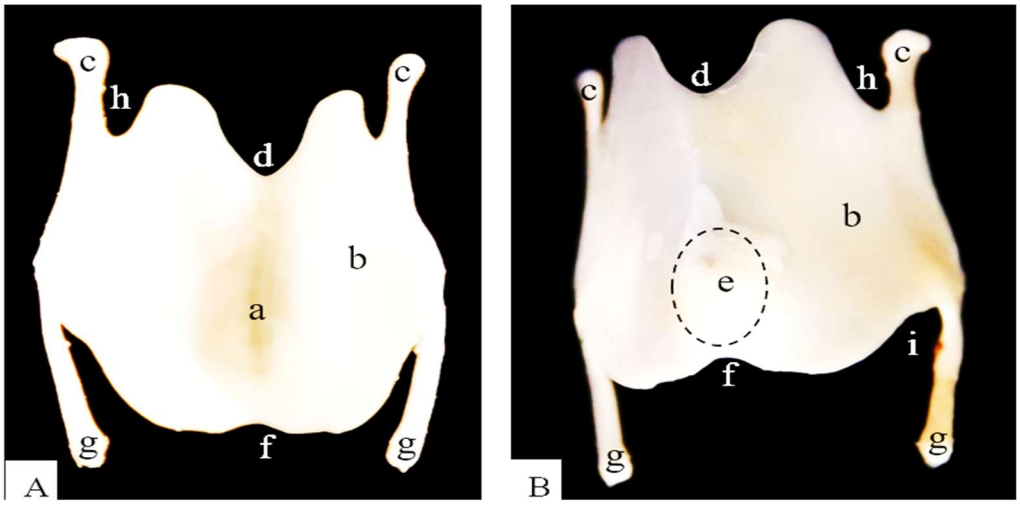

Figure 2: A-B. Thyroid cartilage of Yankasa sheep; A- dorsal view; B- ventral view showing; (a) junction of thyroid laminae, (b) thyroid lamina, (c) rostral cornua, (d) rostral thyroid notch, (e) laryngeal prominence, (f) caudal thyroid notch, (g), caudal cornua, (h) rostral dorsal fissure, (I) caudal dorsal fissure.

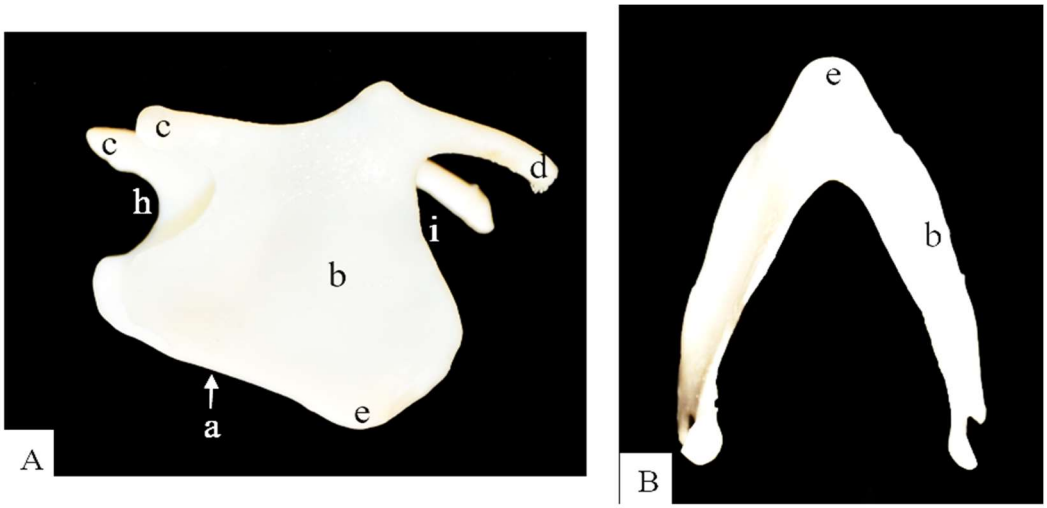

Figure 3: A-B. Thyroid cartilage of Yankasa sheep; A- lateral view; B- caudal view showing; (a) external junction of thyroid laminae, (b) thyroid lamina, (c) rostral cornua, (d) caudal cornua, (e) laryngeal prominence.

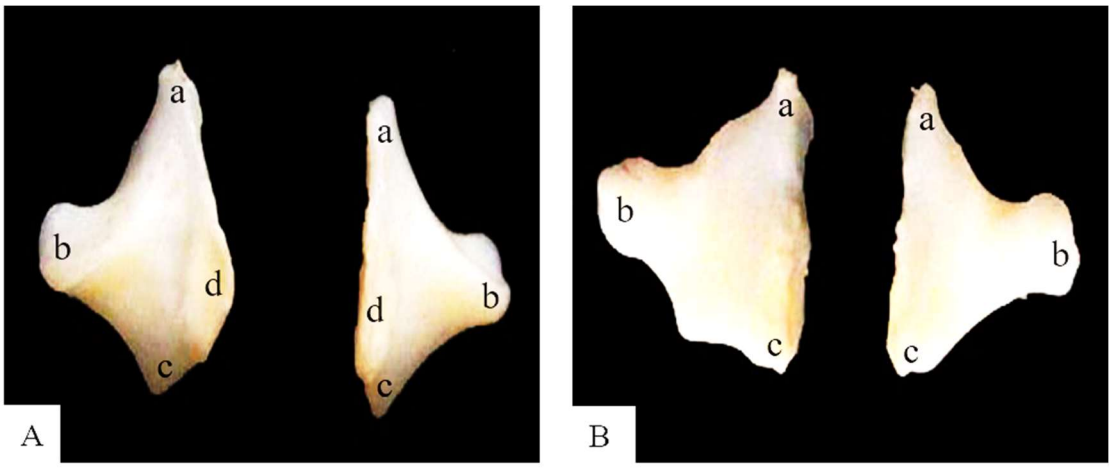

Figure 4: A-B. Arytenoid cartilage of Yankasa sheep; A- rostral view; B- caudal view; C- caudal view showing; (a) corniculate process, (b) muscular process, (c) vocal process, (d) caudal artilcular facet.

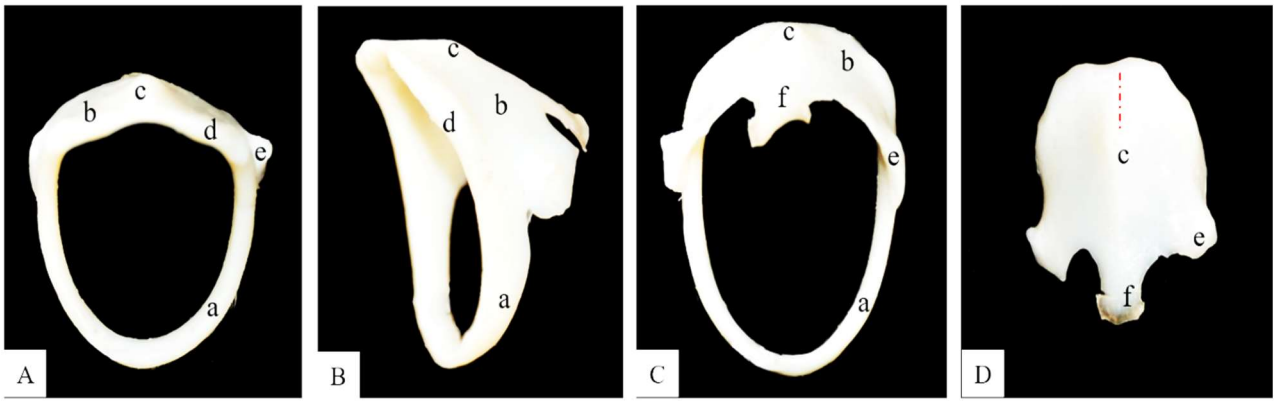

Figure 5: A-D. Cricoid cartilage of Yankasa sheep; A- rostral view; (B) rostrolateral view; (C) caudal view; (D) dorsal view showing; (a) cricoid arch (left), (b) cricoid lamina, (c) median crest, (d) cranial articular surface for arytenoid cartilage, (e) caudal articular surface for thyroid laminae, (f) caudal process.

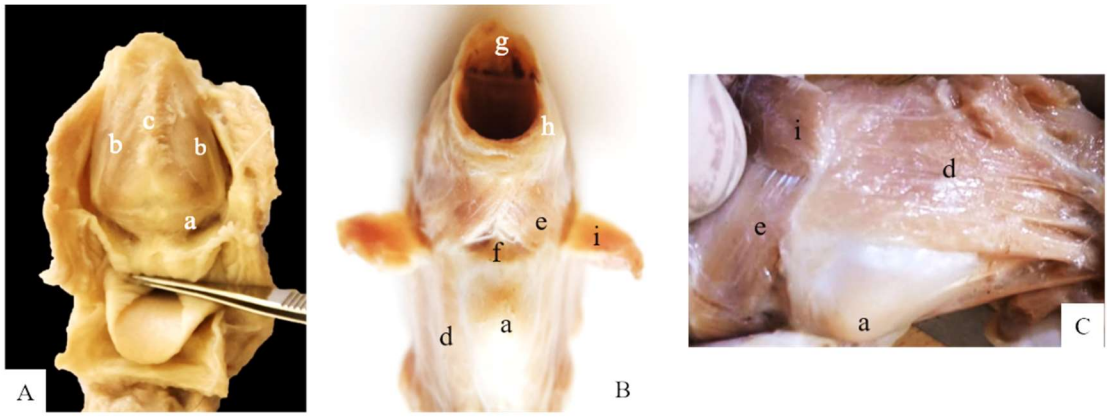

Figure 6: A-C. Larynx of Yankasa sheep; (A) dorsal view. (B) ventral view. (C) lateral view showing; a transverse arytenoid muscle, (b) dorsal cricoarytenoid muscle, (c) median tendinous intersection, (d) thyroarytenoid muscle, (e) cricothyroid muscle, (f) cricothyroid ligament, (g) cricoid lamina caudal border, cricoid arch, (i) thyrohyoid muscle.

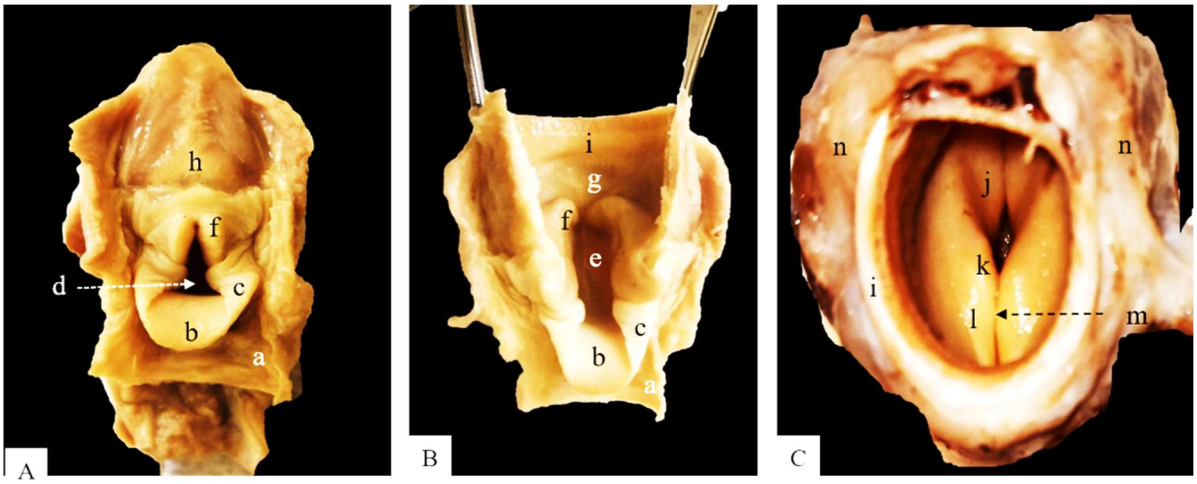

Figure 7: A-C. Larynx of Yankasa sheep; (A) rostro-dorsal view. (B)-Dorsal (laryngeal cavity opened) view. (C) showing; (a) thyrohyoid, (b) epiglottis, (c) aryepiglottic fold, (d) laryngeal inlet, (e) glottic cleft, (f) corniculate tubercle of arytenoid (left), (g) infraglottic cavity, (h) cricoid lamina, (i) first tracheal ring, (j) arytenoid cartilage, (k) vocal process of arytenoid, (l) vocal fold, glottis cleft, (n) thyroid gland.

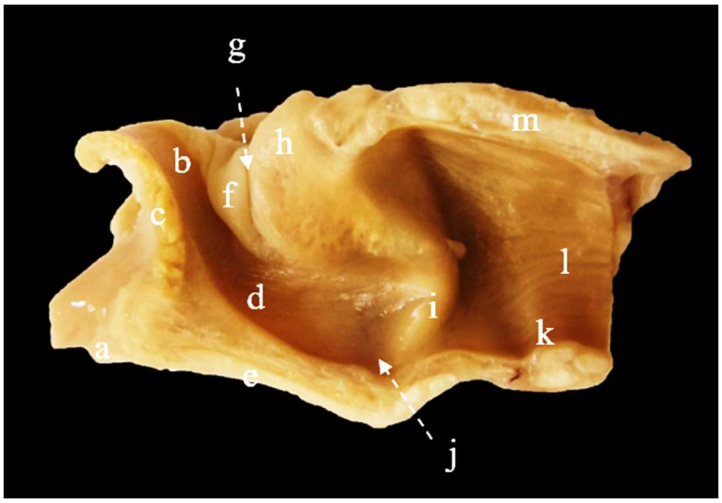

Figure 8: Larynx of Yankasa sheep (Median section with the lining mucosa) showing; (a) hyoepiglotticus, (b) epiglottis, (c) epiglottic cartilage, (d) median laryngeal recess, (e) thyroid cartilage, (f) vestibular fold, (g) slit-like entrance into lateral laryngeal ventricle, (h) corniculate process of arytenoid, (i) vocal fold, (j) depression rostral to vocal fold, (k) cricoid arch, (l) cricotracheal ligament, (m) cricoid lamina.

Histological Findings

The epiglottis was lined by a stratified squamous keratinized epithelium. The free surface of this epithelium was linear devoid of undulation. The epithelium was comprised of the *stratum basale*, *stratum granulosum* and *stratum cornium*. The subepithelial portion of the propria submucosa was slightly dense, whereas the deeper part became loose, consisting of connective tissue cells mainly fibroblast. At some place within the deeper portion of the propria mucosa, a few clusters of fat cells, glandular tissue were observed immediately adjacent the cartilage matrix. The elastic cartilage presented chondrocytes of varying sizes. The chondrocytes especially towards the periphery of the matrix were smaller sized, having flat nucleus and their lacunae were elliptical. The deeper part of the cartilage matrix presented large size chondrocytes with a centrally placed oval nucleus. Some of the lacunae were multicellular, having two or more cells. The pericellular matrix were more defined surrounding the deeply placed chondrocytes (Figure 9).

The thyroid cartilage was lined by pseudostratified columnar epithelium. The epithelium was undulating, presenting folds and crypts at intervals. The subepithelial part of the propria comprised of loose irregular connective tissue, whereas the deeper part of the connective tissue became slightly dense. At some places, elastic fibers were seen in the subepithelial part arranged parallel to the surface epithelium. Fine blood vessels of varying sizes and elastic cartilage, skeletal muscle was observed towards the deeper parts. The hyaline cartilage of the thyroid was surrounded by perichondrium which consists of a cellular layer adjacent to the cartilage and outer fibrous layer (Figure 10).

The paired arytenoid cartilages were lined by stratified squamous non-keratinized epithelium. The free surface of the epithelium was straight whereas the basal surface presented papillae. The epithelium consisted of *stratum basale, spinosum*, *granulosum*, and *stratum cornium*. The propria submucosa comprise of loose irregular connective tissue having connective tissue cells and fibers, whereas clusters of fat cells, fine blood capillaries were observed towards the deep potion of the propria. The hyaline cartilage was surrounded by perichondrium and the core matrix comprises of chondrocytes of different sizes within a lacuna. A few multicellular lacunae were observed especially towards the deeper portion of the cartilage matrix. At some places, cartilage canal comprising of arterioles, venule and capillaries of varying sizes were observed adjacent the cartilage (Figure 11).

The cricoid cartilage was lined by pseudostratified columnar epithelium with goblet cells. The propria mucosa comprised of loose irregular connective tissue having connective tissue cells and fibers. At some places, the subepithelial part of this propria was having slightly dense connective tissue. The propria submucosa also presented few glandular tissues and their ducts, and linearly arranged cavernous veins. The deeper layer presents cartilage and muscle fibers. The cartilage was surrounded by perichondrium which present both the fibrous and cellular layer. At some places, nerves fascicles were observed between the cartilage and deeply laying smooth muscle. (Figure 12).

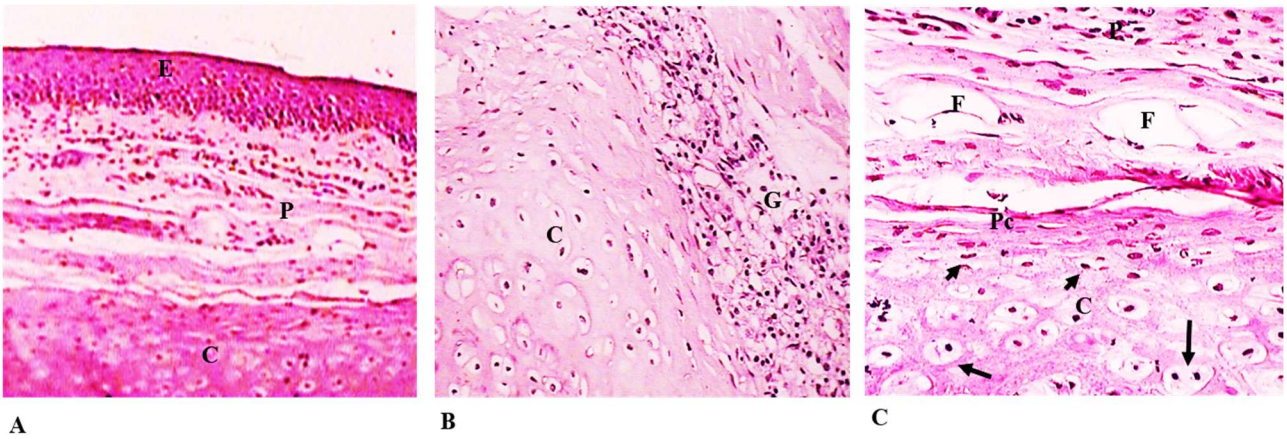

Figure 9: Photomicrograph of epiglottic cartilage showing; A. stratified squamous keratinized epithelium (E) propria submucosa (P) elastic cartilage (C) H. & E. x 100; B. glandular tissue (G) elastic cartilage (C) H. & E. x 100; C. higher magnification of cartilage matrix showing smaller sized chondrocytes (short arrow), multicellular lacunae (long arrow), cluster of fat cells (F) H. & E. x 400.

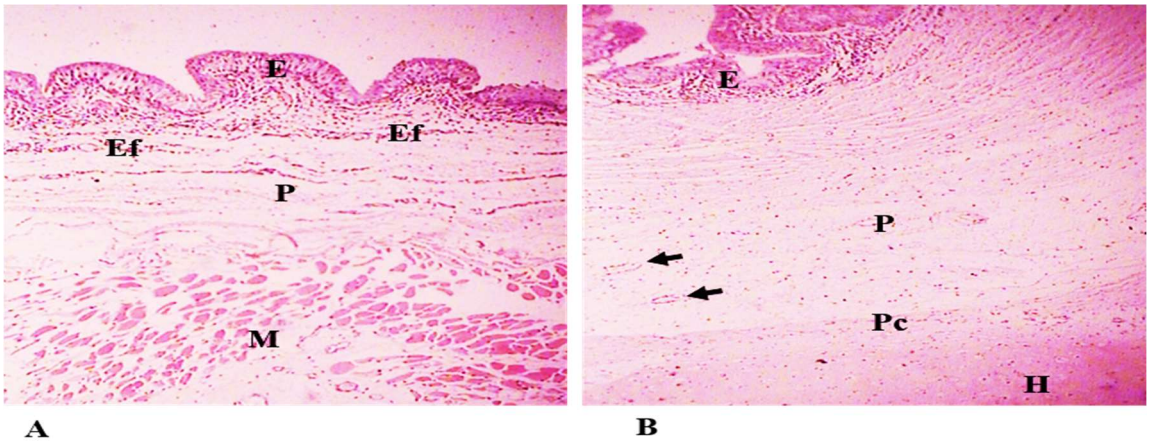

Figure 10: Photomicrograph of thyroid cartilage showing; pseudostratified columnar epithelium (E) propria submucosa (P) elastic fibers (Ef) skeletal muscle (M) H. & E. x 100; B. fine blood vessels (**short arrows**) fibrous layer of perichondrium (Pc) hyaline cartilage (H) H. & E. x 100.

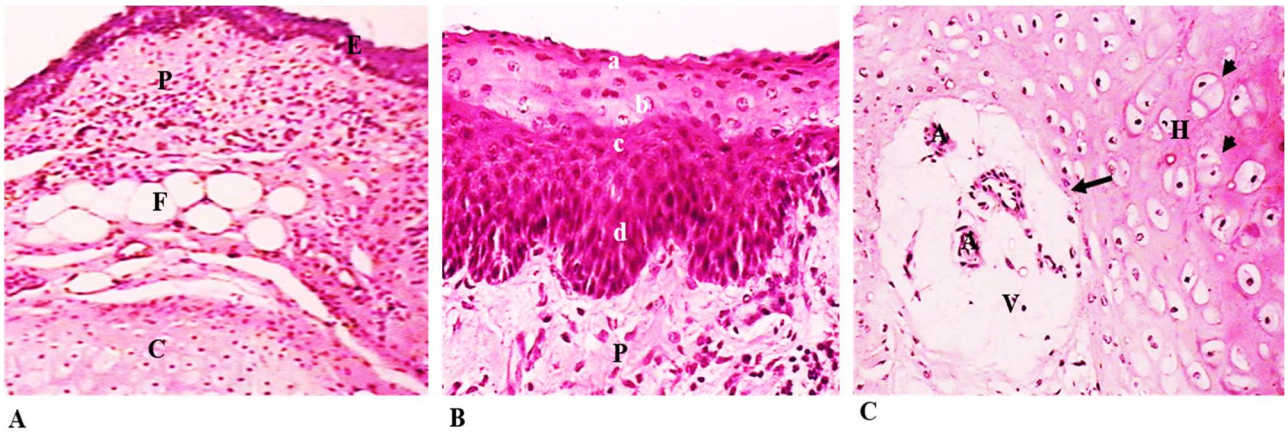

Figure 11: Photomicrograph of arytenoid cartilage showing; A. stratified squamous non-keratinized epithelium (E) propria submucosa (P) cluster of fat cells (F) elastic cartilage (C) H. & E. x 100; B. higher magnification of the surface epithelium showing stratum cornium (a) stratum granulosum (b) stratum spinosum (c), stratum basale (d) basal H. & E. x 400; C. higher magnification of cartilage matrix showing cartilage canal (long arrow) with arteriole (A) venule (V), multinucleated chondrocytes (short arrow) H. & E. x 400.

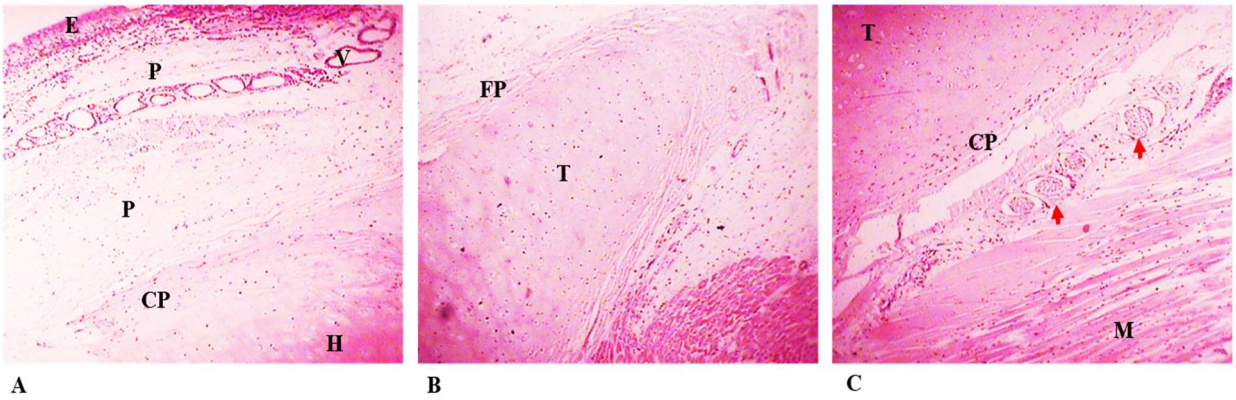

Figure 12: Photomicrograph of cricoid cartilage showing; A: pseudostratified columnar ciliated epithelium (E), propria submucosa (P), cavernous vein (V), secretory ducts (D) H. & E. x 100; B. interterritorial matrix of hyaline cartilage (T), fibrous layer of perichondrium (FP), H. & E. x 100; C. cellular layer of perichondrium (CP), nerve fascicles (red arrows) adjacent epimysium, smooth muscle fibers (M) H. & E. x 100.

DISCUSSION

The slight variation in morphology of laryngeal cartilages, and even the number of the minor elements among species are of great practical significance (Dyce *et al*., 2010). The shape of the free surface of the epiglottic cartilage was variable, described as orbiculate leaf-like in pig (Parkash and Kumar, 2021), oblanceolate leaf like in buffalo, and panduriform leaf in camel (Eshra *et al*., 2016). These leaf-like structures tilted backward to partially cover the entrance to the larynx during swallowing (Dyce *et al*., 2010). The stalk at the base of the epiglottis was in the form of a pointed narrow stalk in donkey (Eshra *et al*., 2016) short and curved rostral in the pig (Parkash and Kumar, 2021). The stalk is embedded between the root of the tongue, the basihyoid, and the body of the thyroid cartilage and attached to all of these structures in domestic mammals (Dyce *et al*., 2010).

Many of the observable features of thyroid cartilage in the present study had earlier been reported in camel, buffalo and donkey (Eshra *et al*., 2016). A rostral horn was absent in giraffe (Erdogan and Perez, 2013). In addition to lacking rostral horn, thyroid fissure, thyroid foramen and laryngeal prominence were also reported to be absent in the pig (Wysocki *et al*., 2010; Parkash and Kumar, 2021). In buffaloes, a permanent thyroid foramen located caudal to the thyroid fissure and a secondary thyroid foramen were observed (Eshra *et al*., 2016). The ventral part of the thyroid cartilage is reduced to a narrow bridge in the horse, in which a large, forward-pointing notch provides a convenient route of entry for laryngeal surgery (Konig and Liebich, 2004). The irregularly pyramidal-shaped outline of the paired arytenoid cartilage and the resultant processes was consistent in domestic mammals. However, the apex of arytenoids did not show well defined corniculate process in Gaddi goat (Baltoo *et al*., 2018) whereas, in giraffe, the presence of a thick arcuate crest that separated lateral arytenoid surface into two different regions for muscles attachment was observed (Erdogan and Perez, 2013). The cricoid cartilage was signet ring shaped with expanded dorsal laminae that narrowed to form arches ventrally. This observation was consistent with the description of cricoid cartilage reported by (Rajani *et al.* (2019) in domestic animals.

The intrinsic muscles of the larynx act on elastic systems of laryngeal ligaments causing dilatation or constriction of the glottis and the attendant changes in position of the vocal folds, thus affecting the movement of the laryngeal cartilages (Nickel *et al*., 1979). The dorsal cricoarytenoid muscle acts as dilators of the glottis cleft (Konig and Liebich, 2004). Some superficial fibers of this muscle in buffalo and donkey's blends with the esophageal wall to form cricoesophageal muscle (Metwally *et al*., 2018). The fan-shaped thyroarytenoid muscle was undivided in Yankasa sheep, similar to observation of Eshra *et al*. (2016) in buffaloes and camel. However, it was divided into a rostral ventricularis and a caudal vocalis which occupy the vestibular and vocal fold in the horse, dog (Dyce *et al*., 2010), giraffe (Erdogan and Perez, 2013) and donkey (Eshra *et al*., 2016). The cricothyroid muscle inserts on the caudal border of the left and right thyroid laminae in yankasa sheep. Whereas in buffaloes, camel and donkeys, the muscle inserted at the ventral border of the caudal thyroid cornua. It has been stated that this muscle tenses the vocal fold and is the only laryngeal muscle innervated by the cranial laryngeal nerve (Konig and Liebich, 2004).

The laryngeal inlet in the Yankasa sheep presented triangular-shaped outline similar to that reported in the camel (Eshra *et al*., 2016). The vestibular fold observed in this study was similarly demonstrated in camels and donkeys (Eshra *et al*., 2016). The vestibular fold is especially prominent in the horse and receive more attention lately (Dyce *et al*., 2010). The opening into the lateral laryngeal ventricle was elliptical-like orifice in giraffe (Erdogan and Perez, 2013). The vocal fold in the pig is split into two portions with small lateral laryngeal ventricle between them (Konig and Liebich, 2004). The median laryngeal recess observed in the present study, had paired characteristic orifices in the donkey leading into small expanded pouches (Eshra *et al*., 2016).

The stratified squamous keratinized epithelium of the epiglottis observed in our study, was non-keratinized and modified at certain places into reticular epithelium in the pig (Parkash and Kumar, 2019). Similarly, the stratum spinosum reported in the pig was absent in our findings. Numerous taste buds were found in the non-keratinized epithelium that lined the base of the epiglottis in buffaloes (Eshra *et al*., 2016), these were not observed in this study. The thyroid and cricoid cartilages were lined by pseudostratified columnar epithelium with goblet cells as reported in the pig (Parkash and Kumar, 2019). In buffaloes, camels and donkeys, the laryngeal epithelium from the base of epiglottis and entire parts caudal to vocal folds were lined by pseudostratified columnar ciliated epithelium with goblet cells (Eshra *et al*., 2016). The paired arytenoid cartilage epithelium was non-keratinized as reported in the pig (Parkash and Kumar, 2019). However, lymphoid cells infiltration observed in the pig was absent in our findings and the stratum granulosum reported in our finding was absent in the pig (Parkash and Kumar, 2019). Cartilage canals were observed in the elastic cartilage of the arytenoid having arterioles and venules. It has been stated that the canals provide nourishment to a given area, where the arterioles end in capillary glomerulus and chondrocytes adjacent to these glomerulus degenerates presenting a circularly layers of actively dividing cells (Eurell and Frappeir, 2006). The few glandular tissue, clusters of fat cells observed in the propria submucosa were similar to findings by Eshra *et al*. (2016) in large ruminants. The glands were predominantly mucous secreting in camel (Eshra *et al*., 2016), whereas in the pig (Parkash and Kumar, 2019) were mixed showing positive reaction for glycogen, sailomucins, hyaluronic acid, weakly acidic sulfated mucosubstances, acidic and neutral mucopolysaccharide. The variation in reactivity of these glandular cells could be a reflection of diet preference among these species.

Conclusion

It is envisaged that this study highlighted some basic information on gross anatomy of the laryngeal cartilages, intrinsic laryngeal muscles, and histomorphology of the cartilages in Yankasa sheep, which might be valuable for comparative studies as well as clinical anatomy.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors Contributions

IAG designed the research work and interpreted findings. AAA collected, processed and photographed samples. MMK reviewed literatures, prepared and proofread the manuscript. All authors have read and agreed with the content of the final manuscript.

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